FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
0273-0011	To Be Determined 10/089503

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/09594	29-September-2000	30-September-1999

TITLE OF INVENTION
THE PRV-1 GENE AND USE THEREOF

APPLICANT(S) FOR DO/EO/US
PAHL, Heike

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

1. Statement to Support Filing and Submission of Sequence Listing in Accordance With 37 CFR 1.821-1.825; 2. English language translation of the Reply to the Written Notice containing Amended Claims, dated 15-October-2001; 3. Paper copy of the sequence listing (10 sequences); and 4. Postcard

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
To Be Determined 10/089503	PCT/EP00/09594	0273-0011

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	11 - 20 =	0	x $18.00	**$0.00**
Independent claims	8 - 3 =	5	x $84.00	**$420.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS =			**$1,440.00**
☐	Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.			**$0.00**
	SUBTOTAL =			**$1,440.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**
	TOTAL NATIONAL FEE =			**$1,440.00**
☐	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			**$0.00**
	TOTAL FEES ENCLOSED =			**$1,440.00**
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$1,440.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0622** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

SHANKS & HERBERT
TransPotomac Plaza
1033 North Fairfax Street, Suite 306
Alexandria, VA 22314
(703) 683-3600
(703) 683-9875 (facsimile)

SIGNATURE

Toni-Junell Herbert
NAME

34,348
REGISTRATION NUMBER

3/29/02
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: PAHL

Art Unit: To Be Determined

Appl. No.: To Be Determined (U.S. National Stage of PCT/EP00/09594)

Examiner: To Be Determined

Atty. Docket: 0273-0011

Filed: Herewith

International Filing Date: September 29, 2000

For: The PRV-1 Gene and Use Thereof

STATEMENT TO SUPPORT FILING AND SUBMISSION OF SEQUENCE LISTING IN ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;

2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same as required by 37 C.F.R. § 1.821(f); and

3. all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

SHANKS & HERBERT

By: _____
Toni-Junell Herbert
Reg. No. 34,348

Date: 3/29/02

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: PAHL, Heike	
	Art Unit: To Be Assigned
Appl. No.: 10/089,503 (U.S. National Stage of PCT/EP00/09594)	Examiner: To Be Assigned
Filed: March 29, 2002 (International Filing Date: Sept. 29, 2000)	Atty. Docket: 0273-0011
For: **The PRV-1 Gene and Use Thereof**	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants wish to thank Mr. Alverado of the PCT branch for his assistance during the teleconference today, March 5, 2003. During that conversation, Mr. Alverado requested that this preliminary amendment be filed to clarify the status of the claims. Specifically, because the amendment dated April 29, 2002 may have caused some confusion as to the status of the claims, applicants specifically request that the April 29, 2002 amendment **not be entered**. In its place, this preliminary amendment clarifies that 11 claims, now numbered 25-35, are pending in the application. Prior to examination, applicants respectfully request that the following amendment be entered:

IN THE CLAIMS:

Please cancel claims 1-24.

Please add the following new claims:

25. An isolated N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof containing at least 50 amino acids; or a biologically active variant thereof.

26. A method for treating pancytopenias and pancytophathies in the bone marrow or blood of a patient, comprising administering to a patient in need thereof an N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof containing at least 50 amino acids; or a biologically active variant thereof.

27. A method for treating pancytopenias and pancytophathies in the bone marrow or blood of a patient, comprising administering to a patient in need thereof an N-glycosylated polynucleotide comprising one of the following nucleotide sequences:
nucleotides 1-1600 of SEQ ID NO:1;
nucleotides 36-1346 of SEQ ID NO:1;
nucleotides 36-1262 of SEQ ID NO:1;

nucleotides 36-1238 of SEQ ID NO:1;
nucleotides 39-1346 of SEQ ID NO:1;
nucleotides 39-1262 of SEQ ID NO:1;
nucleotides 39-1238 of SEQ ID NO:1;
nucleotides 99-1346 of SEQ ID NO:1;
nucleotides 99-1262 of SEQ ID NO:1;
nucleotides 99-1238 of SEQ ID NO:1;
or a biologically active fragment thereof; or a biologically active variant thereof; wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment or variant thereof and wherein the patient cell(s) express an effective amount of the PRV-1 polypeptide or functional fragment or variant thereof.

28. A method for multiplying endogenous cells and/or established cell lines *ex vivo* or *in vitro*, comprising contacting the cells and/or cell lines with an effective amount of an N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a biologically active variant thereof.

29. A method of inhibiting cell growth *in vivo* or *in vitro*, comprising contacting a cell(s) with a cell growth inhibiting amount of an N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;

amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a biologically active variant thereof.

30. The method of claim 29, wherein the polypeptide functions as a cytostatic agent.

31. A method for treating a proliferative disease in a patient, comprising administering to a patient in need thereof an N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a biologically active variant thereof.

32. The method of claim 31, wherein the proliferative disease is selected from the group consisting of: a myeloproliferative disease, polycythemia rubra vera, essential thrombocythemia, myelofibrosis, CML, leukemia, a lymphoma and a solid tumor.

33. A method of inhibiting cell growth, comprising contacting a cell(s) with a polynucleotide comprising one of the following nucleotide sequences:
nucleotides 1-1600 of SEQ ID NO:1;
nucleotides 36-1346 of SEQ ID NO:1;

nucleotides 36-1262 of SEQ ID NO:1;
nucleotides 36-1238 of SEQ ID NO:1;
nucleotides 39-1346 of SEQ ID NO:1;
nucleotides 39-1262 of SEQ ID NO:1;
nucleotides 39-1238 of SEQ ID NO:1;
nucleotides 99-1346 of SEQ ID NO:1;
nucleotides 99-1262 of SEQ ID NO:1;
nucleotides 99-1238 of SEQ ID NO:1;

or a biologically active fragment thereof; or a biologically active variant thereof; wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment or variant thereof and wherein the cell(s) express a growth inhibiting amount of the PRV-1 polypeptide or functional fragment or variant thereof.

34. A method for treating a proliferative disease in a patient, comprising administering to a patient in need thereof an N-glycosylated polynucleotide comprising one of the following nucleotide sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;

or a biologically active fragment thereof; or a biologically active variant thereof; wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment or variant thereof and wherein the patient cell(s) express an effective amount of the PRV-1 polypeptide or a functional fragment or variant thereof.

U.S. Appl. No: 10/089,503
(U.S. National Stage of PCT/EP00/09594)
Atty. Dkt. No: 0273-0011

35. The method of claim 34, wherein the proliferative disease is selected from the group consisting of: a myeloproliferative disease, polycythemia rubra vera, essential thrombocythemia, myelofibrosis, CML, leukemia, a lymphoma and a solid tumor.

REMARKS

Claims 25-35 are pending in the above-identified application. Claims 1-24 have been canceled. Support for new claims 25-35 is found in canceled claims 1-24 and in the specification (English translation) at, for example, pages 12-14. No new matter has been added.

Applicant respectfully asserts that the application is now in condition for examination.

Respectfully submitted,

SHANKS & HERBERT

By: _____
Shelly Guest Cermak
Reg. No. 39,571

Date: 3-5-03

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Pahl, Heike

Art Unit: To Be Assigned

Serial No. 10/089,503 (U.S. National Stage of PCT/EP00/09594

Examiner: To Be Assigned

Filed: March 29, 2002 (International Filing Date: Sept. 29, 2000

Atty. Docket: 0273-0011

For: The PRV-1 Gene and Use Thereof

AMENDMENT AND SUBMISSION OF SEQUENCE LISTING UNDER 37 C.F.R. § 1.825(a)

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. § 1.825(a), Applicants submit substitute sheets to amend the paper copy of the Sequence Listing.

In the Specification:

Please cancel the existing Sequence Listing for the above-identified application, and replace it with the substitute sheets appended hereto.

REMARKS

Applicants' Attorney hereby states that the submission, filed in accordance with 37 C.F.R. § 1.825(a), does not include new matter. Applicants' undersigned attorney has

amended the specification only to direct the entry of this corrected Sequence Listing between the specification and the claims of the above-identified application.

In accordance with 37 C.F.R. § 1.825(b), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same.

Support for the new Sequence Listing is found in the canceled Sequence Listing found between the specification and claims of the above-identified application.

This amendment and submission is totally responsive to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US). The Notice also requests an Oath or Declaration of the inventors. The Oath and Declaration were filed in the United States Patent and Trademark Office on April 20, 2002.

It is respectfully believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

SHANKS & HERBERT

By: _____
Toni-Junell Herbert
Reg. No. 34,348

Date: 9/20/02

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2/prts

The gene PRV-1 and its use

Description

The invention relates to a nucleotide sequence which encodes the PRV-1 gene, to recombinant DNA which contains this nucleotide sequence, to vectors which contain the recombinant DNA and to cells which are transformed with these vectors, and also to a PRV-1 polypeptide, to antibodies against this polypeptide, to a process for detecting the PRV-1 polypeptide and to drugs which comprise the PRV-1 polypeptide or antibodies which are directed against the PRV-1 polypeptide.

Polycythemia rubra vera (erythremia), also termed polycythemia vera or p. vera, is a malignant hematological disease in which there is an increased formation of erythroid, granulocytic and megakaryocytic cells. The disease is of clonal origin and arises as a result of the mutation of a single hematopoietic precursor cell. In Germany, the incidence of p. vera is from 4 to 6 per million inhabitants. If left untreated, the disease leads to death within 18 months. Treatment by means of blood-letting or chemotherapy extends the average survival time to more than 13 years.

P. vera is diagnosed by means of clinical criteria. The clinical picture includes headaches, pruritus, splenomegaly in two thirds of the patients, bleeding or thromboses, hypertension in a third of the patients, gout, which is brought about by an increase in the production of uric acid, and, in some cases, septic ulcers. The most important laboratory finding is an increase in the values for hemoglobin, hematocrit, erythrocyte count and total erythrocyte volume, and also a neutrophilic granulocytosis or thrombocytosis in many cases. Since, on the one hand, most of the criteria are rather diffuse and, on the other hand, not all the patients fulfill these criteria, it is

frequently difficult to distinguish p. vera from other myeloproliferative diseases, such as chronic granulocytic leukemia or essential thrombocytosis, and thereby confirm the diagnosis. To date, the molecular cause of p. vera is completely unknown. Since, however, p. vera takes a severe course if it is not treated, accurate diagnosis is important.

An object of the invention was therefore to find the molecular cause of polycythemia rubra vera and to create the possibility of diagnosing it.

This object was achieved by isolating a gene which is expressed specifically in association with p. vera and not in healthy control individuals. This gene is designated the PRV-1 gene (polycythemia rubra vera).

A similar nucleotide sequence is disclosed in International application WO 98/50552.

One part of the subject matter of the invention therefore relates to a polynucleotide which encodes the PRV-1 gene and essentially comprises the sequence ID No. 1. The polynucleotides of the present invention can be single-stranded or double-stranded DNA or RNA. If they are RNA, it is then clear to the skilled person that "U" nucleotides are present in place of "T" nucleotides. "Polynucleotide" is understood as meaning nucleic acids which contain 15 or more nucleotides.

The nucleotide sequence according to the invention is depicted in figure 1. The invention therefore relates to a polynucleotide which corresponds to the sequence shown in figure 1 and also to a polynucleotide whose nucleotide sequence exhibits minor differences. Within the meaning of the present application, minor differences are understood as meaning those sequences in which a few, preferably not more than 50 and particularly preferably not more than 25, nucleotides can be exchanged, with, however, the function of the gene encoded by the nucleotide sequence being unaffected. The skilled person is familiar with the fact that a base triplet encoding an amino acid can be replaced with another triplet which encodes the same

amino acid. In addition to this, regions which are of less importance can be deleted and/or mutated to a minor extent. In a particular embodiment, the polynucleotide comprises nucleotides 36 to 1346 of sequence No. 1, that is the coding region of the PRV-1 gene. Other embodiments comprise nucleotides 36 to 1262 or 36 to 1238 of sequence No. 1. This region presumably encodes the active region of the PRV-1 polypeptide. Finally, the polynucleotide of the invention can also comprise nucleotides 39 to 1346, 39 to 1262 or 39 to 1238 of sequence No. 1, such that the codon which encodes the starting methionine is not present. A preferred embodiment is a polynucleotide which comprises nucleotides 99 to 1346, 99 to 1262 or 99 to 1238 of sequence No. 1. This results in the codons at the 5' end which encode the signal peptide of the PRV-1 polypeptide not being present.

The polynucleotide according to the invention can also be a fragment of the PRV-1 gene. As a rule, the fragment possesses more than 100 nucleotides, preferably, however, more than 300 nucleotides. The fragments can also be used as primers or as probes, in particular for PCR; in this case, the fragments can be truncated to fit the purpose. Usually, primers have a length of between 10 and 30 nucleotides and probes have a length of between 15 and 50 nucleotides.

The PRV-1 gene is an endogenous gene whose expression in healthy individuals is, however, restricted to only a few organs. Normally, it is expressed in the main in the hematopoietic organs, i.e. in bone marrow and fetal liver, and weakly expressed in the spleen, but not expressed in heart, muscle, pancreas or kidney. In patients who are suffering from p. vera, this gene is very strongly overexpressed in the hematopoietic cells, in particular.

The PRV-1 gene encodes a protein which exhibits the protein sequence shown in Figure 2. The signal peptide, which is present in the protein sequence of all surface molecules and normally removed when the

protein is processed, is divided off by a hyphen. The protein has the sequence ID No. 2. Another aspect of the invention is consequently an essentially pure polypeptide having the sequence No. 2 or a polypeptide having the sequence No. 2 but lacking the signal peptide (i.e. amino acids 22 to 437 of sequence No. 2). Other embodiments encompass amino acids 1 to 409, 22 to 409, 1 to 401 or 22 to 401 of sequence No. 2 (what is probably the active region of the protein).

With regard to biological activity, the polypeptide according to the invention is preferably glycosylated; it is most preferably N-glycosylated. It can then be glycosylated at at least one of the amino acids Asn-46, Asn-189 and Asn-382 of the PRV-1 polypeptide (the amino acid numbers refer to the sequence No. 2). The invention also encompasses fragments of the polypeptides according to the invention which are N-glycosylated. The fragments are at least 50 amino acids in length, preferably at least 100 amino acids and most preferably at least 150 amino acids. In another embodiment, a polypeptide can be O-glycosylated.

It is clear to the skilled person that particular amino acids can be replaced with other amino acids without impairing the biological activity of the protein. Such modified forms of the polypeptides according to the invention are also part of the subject matter of the invention (variants). The amino acid replacements are those which do not have a negative effect on the biological activity of the protein. The skilled person can make use of well known rules for selecting the replacements.

Depending on the method of preparation, the PRV-1 polypeptide can, for example, possess a glycosyl phosphatidylinositol anchor. This is then bonded to the amino acids which correspond to amino acids 407 to 409 in sequence ID No. 2. A GPI anchor is used to anchor a protein by means of a lipid on the outside of the cell membrane. However, for reasons which have not so far

been conclusively elucidated, it is frequently observed that GPI-linked proteins are also released into the medium. This is referred to as "shedding". To date, it has not been clarified whether this is a specific process, i.e. such proteins are cleaved from the membrane by enzymes in a controlled manner, or whether it represents a non-specific loss of the anchor. It is consequently very probable that PRV-1 is to be found both on the cell membrane and extracellularly. The secreted form, which is not membrane-bound, is probably more important for the effect of the polypeptide as a growth factor and growth inhibitor since, as a growth factor, this form is able to diffuse and reach other cells.

It is clear to the skilled person that he can influence the attachment of the protein to the cell membrane by manipulating these C-terminal amino acids. This particularly concerns the preparation of defined DNA constructs which are intended for expressing the PRV-1 polypeptide or fragments of this polypeptide. The codons which encode these amino acids can be mutated or deleted.

The gene encodes a surface receptor of the uPAR/Ly6 family. This receptor family can transduce mitogenic signals, i.e. signals which stimulate cell division. It is therefore assumed that overexpression of the PRV-1 gene, inter alia on the bone marrow cells of p. vera patients, contributes to hyperproliferation of these cells.

It has been found that PRV-1 is not expressed on granulocytes in healthy individuals or in patients suffering from other myeloproliferative diseases, e.g. suffering from chronic granulocytic leukemia, acute granulocytic leukemia, essential thrombocytosis or secondary erythrocytosis.

In order to be able to use the polypeptide encoded by the PRV-1 gene for analyses and detection methods, it is expediently generated from recombinant DNA, with the recombinant DNA preferably comprising the

nucleotide sequence ID No. 1 or at least the coding region of the PRV-1 gene, that is nucleotides 36 to 1346 of sequence ID No. 1, or else at least nucleotides 39 to 1262 or 39 to 1238, functionally linked to a promoter. However, the recombinant DNA can also comprise only a fragment of sequence No. 1.

The invention furthermore relates to a vector which contains the recombinant DNA for the PRV-1 polypeptide, or a fragment thereof, and to a host cell which is transfected or transformed with this vector. The host cells may be prokaryotic, for example bacteria such as E. coli. However, the polypeptides which are expressed are then not glycosylated. Preference is therefore given to eukaryotic host cells, which are able to glycosylate the expressed protein post-translationally and modify it in other ways. Examples of eukaryotic host cells are insect cells, such as Sf9 cells, for expression following infection with recombinant baculoviruses, and mammalian cells, such as 293 cells, COS cells, CHO cells and HeLa cells. These examples are not exhaustive. It is also possible to use yeast cells as host cells. It is clear to the skilled person that the glycosylation pattern can differ depending on the host cell. The biological activity of the expression product can therefore also vary. Particular preference is given to host cells which glycosylate the expression product in such a way that the biological activity of the protein is retained.

Another aspect of the invention is a process for preparing a polypeptide according to the invention. In this process, a DNA encoding the polypeptide according to the invention is caused to be expressed in a host cell. The culture medium or the cells is/are employed for the subsequent isolation of the polypeptide depending on whether the expressed polypeptide is secreted by the host cell into the culture medium or remains in the cell. After that, the polypeptide according to the invention is concentrated and/or purified using methods which are known in the

state of the art, for example chromatographic methods. Methods for purifying proteins are described, for example, in Scopes, R., Protein Purification: Principles and Practice (3rd edition), Springer Verlag (1994). In one particular embodiment, the process according to the invention encompasses the step in which glycosylated polypeptide is concentrated and/or purified. This step can take place either before the polypeptide according to the invention has been essentially purified or after it has already been essentially purified. In the latter case, the glycosylated moiety of the purified polypeptide is then separated off and isolated. In the most preferred embodiment of the process, N-glycosylated polypeptide is specifically isolated. In another embodiment of the process, polypeptide is isolated which is glycosylated at at least one of the amino acids Asn-46, Asn-189 and Asn-382 of sequence No. 2.

The PRV-1 polypeptide which is isolated from granulocytes or produced recombinantly can be employed both for diagnosing polycythemia vera and for treating the disease.

One therapeutic possibility is that of "antisense therapy". This method employs an "antisense" RNA molecule, that is an RNA which is complementary to the PRV RNA. Since the PRV-1 RNA has the sequence 5'-AAAAGCAGAAAGAGATTACCAGCC-3' (seq. ID No. 3) at its beginning, the requisite antisense RNA directed against this sequence would possess the following nucleotide sequence: 5'-GGCTGGTAATCTCTTTCTGCTTTT-3' (seq. ID No. 4). This antisense RNA is incorporated into a vector and introduced into the p. vera cells. This RNA is introduced, for example, by means of transfection, with the vector used for the transfection preferably being configured such that it is introduced specifically into the p. vera cells. Expression of the antisense RNA results in it no longer being possible for the PRV-1 mRNA to be translated into a polypeptide. Cells which

have been treated in this way do not then form any PRV-1 protein.

The invention therefore also relates to a process for detecting p. vera which is characterized in that the PRV-1 polypeptide, or an epitope thereof, is detected and the extent of the expression is determined.

Overexpression of this receptor on mature cells outside of the bone marrow, e.g. on granulocytes, is a strong indication of the presence of the disease p. vera. This overexpression is expediently detected by means of an immunoassay using antibodies which are directed against the PRV-1 receptor. Suitable test methods are the known immunoassay variants which make use of PRV-1 polypeptide-specific antibodies together with other labeled antibodies which can be immobilized or in solution. The labeling can be effected in a manner known per se, for example using radioactive isotopes, by means of fluorescence or luminescence, using enzymes, by means of color-forming reactions or using other groups which are suitable for the determination. These variants are known to the skilled person and do not require any more detailed explanation here. According to the invention, ELISA tests are particularly preferred.

The antibodies which are required for specifically detecting the PRV-1 receptor can likewise be prepared in a manner which is known per se. Both monoclonal and polyclonal antibodies are suitable, with preference being given to using monoclonal antibodies.

Peptides which are derived from the protein can also be used for preparing antibodies. Within the context of the present invention, success was achieved using the peptides having the sequences:

a) KVSDLPRQWTPKN (amino acids 34 to 46) [seq. ID No. 5], and

b) SAREKRDVQPPASQH (amino acids 391 to 405) [seq. ID No. 6].

The polyclonal antibodies are normally produced by immunizing a suitable host (rabbit) with the PRV-1 polypeptide, where appropriate bound to an immunological support (adjuvant), and eliciting an immune response. Monoclonal antibodies can be generated in a manner known per se using the hybridoma technique. The antibodies can be purified by means of affinity purification. The preparation and purification of antibodies are described, for example, in "Antibodies: A Laboratory Manual" by Harlow and Lane, Cold Spring Harbor Laboratory Press.

Furthermore, such polyclonal or monoclonal antibodies which are directed against PRV-1 can also be used for treating the disease.

In another embodiment, the PRV-1 receptor can be detected using an RT-PCR method. For this, RNA is first of all isolated from the PRV-1-overexpressing cells, which are as a rule granulocytes. A reverse transcription is then performed in a manner known per se using an RT primer. The RT primer is preferably a primer which has the following nucleotide sequence (SEQ ID No. 7):

ATTAGGTTATGAGGTCAGAGGGAGGTT.

In this way, the specific PRV-1 RNA is transformed into DNA. This DNA is then amplified in a PCR reaction in a manner known per se. The following two primers are preferably employed for the amplification cycles:

As the sense primer (SEQ ID No. 8)

GCAGAAAGAGATTACCAGCCACAGACGG.

As the antisense primer (SEQ ID No. 9)

GAATCGTGGGGGTAATAGAGTTAGCAGG.

The skilled person is readily able to use the disclosed sequence to find other primers which are also suitable.

Since the RNA is used as the starting material for this method, the PCR signal is only positive when the PRV-1 gene is also expressed. As explained above, this is only the case when the patient is suffering from p. vera. PRV is not expressed in granulocytes of healthy patients. Consequently, the absence of any RT-PCR signal indicates that no p. vera is present. The quantification in the RT-PCR method is preferably effected using the TaqMan® technology. This quantification requires a probe in addition to primers. The preferred sequence of the probe is 5'-TTCTTGTTGAACCACACCAGACAAATCGG-3' (SEQ ID NO:10). Quantitative RT-PCR for detecting the PRV-1 transcript is therefore also part of the subject matter of this invention.

In another alternative, it is also possible to use a blotting method, preferably a Northern Blot, for diagnosing p. vera. For such a method, the RNA is isolated from granulocytes and then examined for the expression of PRV-1 using a blotting method, for example Northern blotting. The cDNA sequence of SEQ ID No. 1, or a segment of the sequence, can be used as the probe. Hybridization then only occurs if the granulocytes are derived from a patient suffering from p. vera since only then is there any expression on the granulocytes. The absence of hybridization indicates that the individual from whom the granulocytes are derived does not have p. vera.

It is also possible to use a fragment of the gene for the Northern blot hybridization. Such a fragment is normally more than 100 bases in length, preferably more than 300 bases in length. Alternatively, various different fragments of the gene,

which can be used as probes in the Northern blot, can be prepared by digesting the gene with restriction endonucleases. If the fragments are derived from the cDNA, they are then present as double strands which have to be separated into the single strands for the hybridization. Suitable examples are the Bam HI-PstI fragment from base pair 420 to base pair 831, or the PstI-PstI fragment from base pair 831 to base pair 1900.

PRV-1 mRNA, and consequently the expression of PRV-1, can also be detected by first of all reverse-transcribing the mRNA in an RT-PCR reaction and then amplifying the cDNA; the amplified DNA is then detected with a probe in a hybridization method.

In the case of a positive diagnosis, the disease has to be treated since it otherwise leads to death within a relatively short period of time. For this treatment, it is possible to use specific antibodies which are directed against PRV-1 and to which cytotoxic components can be bonded, where appropriate.

The invention therefore furthermore relates to a drug which, in addition to the customary excipients, comprises antibodies which are directed against the PRV-1 receptor.

Since the PRV-1 receptor is overexpressed in p. vera, many antibodies are bound on the surface of the affected granulocytes when they come into contact with the anti-PRV-1 antibody. The binding of many antibodies to these cells stimulates the immunological cells to destroy these granulocytes. In this way, it is possible to eliminate the p. vera cells specifically.

Surprisingly, it has also been found that the PRV-1 polypeptide exhibits haematopoietic activity. The PRV-1 polypeptide is able to stimulate certain hematopoietic precursor cells to form erythroid colonies. It is particularly the N-glycosylated PRV-1 polypeptides which display this function. The polypeptides according to the invention which are

preferred are therefore the N-glycosylated PRV-1 polypeptides, and fragments thereof, which display the growth factor activity.

Another aspect of the invention is therefore a drug which, in addition to a pharmaceutically tolerated excipient, comprises the PRV-1 polypeptide or a biologically active fragment thereof. The PRV-1 polypeptide is preferably glycosylated PRV-1 polypeptide and, even more preferably, N-glycosylated PRV-1 polypeptide or a biologically active fragment thereof. The invention also relates to drugs which comprise at least one polynucleotide according to the invention.

The present invention furthermore relates to the use of PRV-1 polypeptide, or a biologically active fragment thereof or a biologically active variant thereof, as a growth factor in vivo and ex vivo. The PRV-1 polypeptide, or a biologically active fragment thereof or a biologically active variant thereof, can be used for treating all pancytopenias and pancytopathies in the bone marrow and in the circulation (change in the cellular constituents of the peripheral blood and bone marrow). The polypeptides of the present invention can, for example, be used for treating anemias in the case of kidney failure, chemotherapy or whole body radiation, for treating neutropenias and thrombocytopenias during chemotherapy or whole body radiation, for the ex-vivo treatment of peripheral or bone marrow stem cells for expansion (multiplication) and retransfusion into the patients, and for treating sepsis, systemic inflammatory response syndrome (SIRS) or regional inflammatory reactions. The polypeptides of the present invention, or drugs which comprise them, can be administered in a wide variety of ways. The forms of administration comprise intravenous, intramuscular, subcutaneous, intraperitoneal, oral, transdermal and transmucosal administration.

The polynucleotides according to the invention can also be used for treating pancytopenias and

pancytopathies. In this case, the aim is to express a PRV-1 polypeptide, or a functional fragment thereof, in cells of the affected patient. Gene therapy methods are first and foremost used in this connection. Cells can be isolated from the patient and transfected with a polynucleotide according to the invention (ex-vivo manipulation), after which they are then returned to the patient. It is also possible to conceive of methods in which the polynucleotides according to the invention gain access into the target cells by means of viral transfer. Expression of the inserted nucleic acids then leads to haematopoietic activity.

Surprisingly, it was also found that, at higher concentration, the PRV-1 polypeptide has an inhibitory effect on the growth of cells. Thus, it was observed, for example, that adding an increased quantity of PRV-1 protein virtually completely stops the formation of erythroid and granulocytic/monocytic colonies. This effect resembles the action of Interferon-α, which is used, inter alia, therapeutically in chronic myeloid leukemia (CML) and in p. vera. An endogenous inhibitory substance possesses great advantages as compared with a chemical cytostatic agent, such as hydroxyurea, which was used when Interferon-α was not yet available and is to some extent still used. A disadvantage of Interferon-α is that this active compound has very severe side effects. The patients feel as if they were suffering from a serious influenza. The present invention makes available a hematopoiesis-inhibiting substance, with the inhibitory activity being concentration-dependent.

Another aspect of the invention is therefore the use of a PRV-1 polypeptide, as described in this application, for inhibiting the growth of cells, in particular its use as a cytostatic agent. Preference is given to the polypeptide being used for inhibiting the growth of hematopoietic cells. The invention also relates to the use of a polypeptide according to the invention for producing a drug for treating

proliferative diseases. These diseases are, in particular, the myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML and also all leukemias and lymphomas and also solid tumors.

Another aspect of the invention is the use of a polynucleotide, as described in this application, of a biologically active fragment or of a biologically active variant thereof, for inhibiting the growth of cells. The polynucleotide can be incorporated into a suitable vector and transfected into suitable target cells. After the PRV-1 polypeptide, or a biologically active fragment thereof, or a biologically active variant thereof, has been expressed in an appropriate concentration, the growth-inhibiting effect comes into operation. In the same way, the polynucleotide can be incorporated into a viral vector, after which appropriate target cells are infected virally, leading to PRV-1 being expressed. The invention also relates to the use of a polynucleotide of this application for producing a drug for treating proliferative diseases, such as the myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML and also all leukemias and lymphomas and also solid tumors.

The invention also relates to kits for detecting either polycythemia vera or disturbances of the hematopoietic system. These kits comprise a polynucleotide according to the invention and/or a polypeptide according to the invention and/or one or more antibodies according to the invention. In addition to this, the kit can also comprise a container or compositions which are suitable for implementing detection reactions. Examples of such compositions are buffer solutions, reagents for blocking membranes, hybridization solutions, secondary antibodies, substrate solutions for detection reactions, etc. The kit is preferably used for implementing PCR reactions, RT-PCR, Northern blots, Southern blots, Western blots and ELISA, RIA or similar reactions.

The following examples are given in explanation.

Example 1

Characterizing the PRV gene

The following experiments were carried out in order to characterize the gene:

- the following protocol was used to isolate granulocytes from stored blood or from blood obtained by bleeding p. vera patients:
- an equal volume of 3% dextran solution in 0.9% NaCl was added to the blood and the mixture was left to stand at room temperature (RT) for 20 minutes.
- The mixture separated into two phases. The upper, light-colored phase was removed and centrifuged for 10 minutes at 1800 g and at RT.
- The supernatant was discarded and the cell pellet was resuspended in the same volume of 0.9% NaCl.
- In each case 35 ml of the cells in NaCl were layered on 15 ml of Ficoll-Hypaque.
- The cells on the Ficoll-Hypaque were then centrifuged for 60 minutes at 1800 g and at RT without using the brake.
- A cell pellet and two layers with an interphase were formed.
- The layers and interphase were aspirated off and the cell pellet was resuspended for 30 seconds in 10 ml of ice-cold 0.2% NaCl, and 10 ml of ice-cold 1.6% NaCl were added immediately after 30 seconds.
- The cells were centrifuged down for 10 minutes at 1800 g and at RT.
- They were then washed once in 10 ml of PBS and centrifuged down.
- The cell pellet contained 95-99%-pure granulocytes.
- RNA was isolated from these cells using standard methods.

- 10 mg of this RNA were examined for the expression of PRV-1 in a Northern blot. The entire cDNA sequence shown in SEQ ID No. 1 was used as a probe.

This experiment was performed on 39 p. vera patients and 29 control samples of stored blood. The PRV-1 probe was found to hybridize strongly in the case of the p. vera patients. No hybridization was observed in healthy control samples.

Example 2

PRV-1 possesses growth factor activity

Embryos were removed from a pregnant mouse 13.5 days after fertilization. The fetal livers were removed. The cells contained in them were stained using antibodies and enriched for particular cells, and depleted for other cell types, by means of column chromatography. This results in a cell mixture which is enriched for certain hematopoietic precursor cells (colony forming units-erythroid, CFU-E). Thus, while in all approximately 2% of the fetal liver consists of CFU-E, 30-40% of the enriched cells consist of CFU-E.

These CFU-Es were transfected using a retrovirus. To do this, a packaging cell line, designated 293-T, was itself transfected 48 hours previously. 293-T cells are an established human embryonic kidney cell line. 293-T cells are stably transfected with several genes from a retrovirus. If these 293-T cells are now transfected with two plasmids, termed pOS and pKAT, the 293-T cells then produce a retrovirus which is able to infect murine fetal liver cells. If the 293-T cells are transfected with an empty pOS vector and pKAT, a wild-type retrovirus, which only expresses retroviral proteins, is then produced. On the other hand, cloning a human gene, e.g. PRV-1, into the pOS vector results in the production of a retrovirus which expresses this protein

when it has infected cells. The 293-T cells secrete the retrovirus into the cell culture medium.

After two days, the cell culture medium from the transfected 293-T cells which contains the retrovirus is harvested and filtered once through a 0.45 μm filter. In order to transfect the fetal liver cells, these latter cells are mixed with the filtered cell culture medium, which contains the retrovirus, and centrifuged for 2 hours at 1800 rpm and 20°C in the added presence of Polybren. The transfected fetal liver cells were then cultured in a medium (Methocult, from Cell Systems) which contains, in addition to the usual salts and amino acids, fetal calf serum, 0.0001-0.4 IU of erythropoeitin (EPO)/ml and methyl cellulose (0.8%). The CFU-Es require EPO in order to form hematopoietic colonies. The methyl cellulose solidifies the medium in the form of a jelly, thereby fixing individual cells in this jelly so that, in contrast to being in a liquid medium, they cannot move. It is therefore possible to observe whether a hematopoietic colony is or is not formed from a single cell. CFU-Es form erythroid colonies, that is colonies which contain red blood cells and their precursor cells.

After three days, a count is taken of the number of hematopoietic colonies which have developed. Various mixtures are compared. The mixtures were not all examined in each experiment; mixtures 1-3 are very similar controls and each of them can be compared individually with mixture 4.

Mixture 1: Cells which were not transfected with a retrovirus;

Mixture 2: Cells which were transfected with an empty pOS vector;

Mixture 3: Cells which were transfected with a "green fluorescent protein" (GFP), a protein which is not hematopoietically active.

Mixture 4: Cells which were transfected with pOS-PRV-1 (vector + gene according to the invention).

Table 1: The table lists the results obtained from three experiments which were performed as described. The figures in each case indicate the number of colonies.

	Mixture 1	Mixture 2	Mixture 3	Mixture 4
	un-transfected	empty vector (pOS)	GFP (pOS-GFP)	PRV-1 (pOS-PRV-1)
Experiment 1	116	156	80	326
Experiment 2		271	273	410
Experiment 3	120		131	291

The experiments demonstrate that CFU-Es which were transfected with PRV-1 form very many more colonies (up to three times as many) than do the various control CFU-Es. This result indicates that PRV-1 is a growth factor for CFU-E.

Example 3

Solubility of the PRV-1 growth factor

A further experiment was carried out in order to investigate whether PRV-1 is a soluble growth factor or whether cell-cell contact is required. It is not only a retrovirus which is produced by the packaging cell line 293-T after it has been transfected with the pOS and pKAT vectors. In addition, the 293-T cells also synthesize the protein encoded by the gene cloned in pOS, i.e. PRV-1 in the present case. If the gene product is a soluble protein, it is secreted into the medium which surrounds the packaging cell line 293-T. If the 293-T cells are transfected only with the pOS

vector, without pKAT, no retroviruses are then formed. The cell culture medium then only contains the soluble protein produced by the cells. Medium which is derived from pOS-PRV-1-transfected cells, and which does not contain any retrovirus, is mixed with CFU-Es and the whole is plated out in the methyl cellulose medium; the resulting colonies are then counted.

The following results were obtained:

Table 2: Solubility of PRV-1. The figures in each case indicate the number of colonies.

	Mixture 1	Mixture 2	Mixture 3	Mixture 4
	un-transfected	empty vector (pOS)	GFP (pOS-GFP)	PRV-1 (pOS-PRV-1)
Experiment 4		137	187	557

In this experiment, too, CFU-Es which were treated with PRV-1-containing medium formed very many more hematopoietic colonies than did control cells. It can be concluded from this result that PRV-1 is a soluble growth factor.

Example 4

PRV-1 also has an inhibitory, cytostatic effect.

The experiments were carried out on peripheral blood cells. Since a small number of precursor cells are also circulating in the peripheral blood in healthy individuals, it is possible to culture hematopoietic colonies from peripheral blood cells in a suitable medium (methyl cellulose). 40 ml of peripheral venous blood were withdrawn from a healthy donor (while initially introducing heparin or EDTA as an anticoagulant). 15 ml of Ficoll/Hypaque were added to the blood and the mixture was centrifuged at 1 600 rpm for 40 minutes without braking. This results in the production of a density gradient which fractionates the

blood into its cellular constituents. After the centrifugation, what are termed the mononuclear cells, which also include the stem cells, are to be found at the interphase between serum and Ficoll. This interphase was removed and washed in PBS (isotonic salt solution). This yields purified mononuclear cells, approximately 0.1% of which are hematopoietic stem cells.

The mononuclear cells were taken up in a particularly rich medium (IMDM) which contained an added concentration of 3% FCS (fetal calf serum). This 3% FCS/IMDM subsequently contained the modifications, i.e. PRV-1 was or was not added to it.

The mononuclear cells in IMDM were added, at a density of 7×10^5 cells/ml, to a commercially available medium supplied by Stem Cell Technologies (Methocult), which contained IMDM and 30% FCS, 1% BSA (bovine serum albumin), mercaptoethanol, 2 mM L-glutamine, 3 IU EPO (erythropoietin)/ml and 1.0% methyl cellulose. The cells grew for 14 days in this medium. The few stem cells which are present in this mixture are able to develop into hematopoietic colonies. Usually, between 100 and 200 hematopoietic colonies develop for every 7×10^5 cells employed.

A cell line which expresses a very high quantity of PRV-1 was also constructed. The PRV-1 which is produced by these cells is altered such that it no longer possesses a lipid anchor. The expression product consists of the amino acids 1-401 of the sequence SEQ ID No:2; the amino acids 402-437 are therefore missing. This altered PRV-1 is therefore not, like wild-type PRV-1, incorporated into the cell membrane by means of a lipid anchor, but is instead completely secreted from the cells. As in example 3, the cell line consisted of 293 cells which do not produce any retrovirus but which express protein (PRV-1).

For the hematopoietic colony assays, the mononuclear blood cells were now taken up either in

IMDM medium, which had been incubated for 48 hours with untransfected cells (293), or in medium which had been incubated for 48 hours with cells which were expressing the altered PRV-1 (293-GPI-less-PRV-1). The ability of these cells to form hematopoietic colonies was then investigated. The number of erythroid (red) and myeloid (white) blood cell colonies was determined after 14 days. The experiment was repeated three times and also carried out on different days and using different blood donors. Duplicates were also evaluated within the experiment. The following results were obtained:

Experiment 1

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies
293	248/221	70/114	127/161	25/66
293-GPI-less-PRV-1	7/3	0/0	31/19	0/0

Experiment 2

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies
293	99/91	20/19	49/33	8/1
293-GPI-less-PRV-1	0/0	0/0	0/0	0/0

Experiment 3

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies

293	107/207	22/30	24/32	5/8
293-GPI-less-PRV-1	4/3	0/6	0/1	3/0

It can be concluded from this data that a higher dose of PRV-1 than that used in example 3 possesses a cytostatic effect.

Example 5

The growth factor PRV-1 is N-glycosylated

Granulocytes were isolated from a patient suffering from p. vera, and protein extracts were prepared from these cells using a standard protocol. These protein extracts were treated in accordance with the protocol for the "N-Glycosidase F Deglycosylation Kit" supplied by Boehringer Mannheim. In detail, this means that a "denaturation buffer" was added to the protein extracts and the mixtures were heated at 95°C for 3 minutes, after which they were treated either with "reaction buffer" or with "reaction buffer" plus N-glycosidase. Each mixture was incubated overnight at 37°C and the proteins were analyzed on a PAGE gel electrophoresis followed by a Western blot. The PRV-1 protein was detected with an antibody directed against a protein having the amino acid sequence ID No. 5. The results show that while PRV-1 protein purified from granulocytes is 60-65 kDa in size, it is only 40 kDa in size after having been digested with N-glycosidase. This clearly proves that PRV-1 is glycosylated on asparagine residues (asparagine = N).

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Patent claims

1. An N-glycosylated polypeptide, essentially comprising one of the following amino acid sequences:
   amino acids 1-437 of sequence No. 2;
   amino acids 1-409 of sequence No. 2;
   amino acids 1-401 of sequence No. 2;
   amino acids 22-437 of sequence No. 2;
   amino acids 22-409 of sequence No. 2;
   amino acids 22-401 of sequence No. 2;
   or a fragment thereof containing at least 50 amino acids.

2. A polypeptide, essentially consisting of one of the following amino acid sequences:
   amino acids 1-409 of sequence No. 2;
   amino acids 1-401 of sequence No. 2;
   amino acids 22-409 of sequence No. 2;
   amino acids 22-401 of sequence No. 2.

3. An antibody against a polypeptide of claim 1.

4. An antibody as claimed in claim 3, characterized in that it is a monoclonal antibody.

5. A process for detecting polycythemia vera, characterized in that the PRV-1 polypeptide is reacted, in an immunoassay, with one or more antibodies as claimed in claim 3 or 4.

6. A process as claimed in claim 5, characterized in that the antibody employed is a polyclonal or monoclonal antibody as claimed in claim 3 or 4.

7. A drug for treating polycythemia vera, characterized in that, in addition to customary

excipients, it comprises antibodies as claimed in claim 3 or 4.

8. A drug which comprises a polypeptide as claimed in claim 1 or a polypeptide which essentially comprises one of the following amino acid sequences:

amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;

and at least one pharmaceutically tolerated excipient.

9. A drug which comprises a polynucleotide which essentially comprises one of the following nucleotide sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;

and at least one pharmaceutically tolerated excipient.

10. The use of a polypeptide as claimed in claim 1 or of a polypeptide which essentially comprises one of the following amino acid sequences:

amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;

amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a biologically active variant thereof, as a growth factor.

11. The use of a polypeptide as claimed in claim 1 or of a polypeptide which essentially comprises one of the following amino acid sequences:
amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a biologically active variant thereof, for producing a drug for treating pancytopenias and pancytopathies in the bone marrow and in the circulation.

12. The use of a polynucleotide which essentially comprises one of the following nucleotide sequences:
nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;
or of a fragment thereof or a variant thereof, for producing a drug for treating pancytopenias and pancytopathies in the bone marrow and in the circulation.

13. The use of a polypeptide as claimed in claim 1 or of a polypeptide which essentially comprises one of the following amino acid sequences:
amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a biologically active variant thereof, for treating and/or multiplying endogenous cells and/or established cell lines ex vivo or in vitro.

14. The use of a polypeptide as claimed in claim 1 or of a polypeptide which essentially comprises one of the following amino acid sequences:
amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a biologically active variant thereof, for inhibiting the growth of cells.

15. The use as claimed in claim 14, characterized in that the polypeptide is used as a cytostatic agent.

16. The use of a polypeptide as claimed in claim 1 or of a polypeptide which essentially comprises one of the following amino acid sequences:
amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;

amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a biologically active variant thereof, for producing a drug for treating proliferative diseases.

17. The use as claimed in claim 16, characterized in that the proliferative disease is selected from the group comprising myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML, all leukemias and lymphomas and also solid tumors.

18. The use of a polynucleotide which essentially comprises one of the following nucleotide sequences:
nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;
or of a fragment thereof or a variant thereof, for inhibiting the growth of cells.

19. The use of a polynucleotide which essentially comprises one of the following nucleotide sequences:
nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;

nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;

or of a fragment thereof or a variant thereof, for producing a drug for treating proliferative diseases.

20. The use as claimed in claim 19, characterized in that the proliferative disease is selected from the group comprising myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML, all leukemias and lymphomas and also solid tumors.

21. A kit for detecting polycythemia vera or disturbances of the hematopoietic system which comprises at least one polynucleotide which essentially comprises one of the following nucleotide sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;

or a fragment thereof or a variant thereof,

and/or

at least one polypeptide as claimed in claim 1 or a polypeptide which essentially comprises one of the following amino acid sequences:

amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;

or a biologically active fragment thereof or a biologically active variant thereof

and/or

at least one antibody as claimed in claim 3 or 4.

22. A kit for detecting the PRV-1 protein as claimed in claim 21, characterized in that it is an ELISA test kit.

23. A kit as claimed in claim 21 for detecting PRV-1 mRNA, characterized in that it is a semiquantitative or quantitative RT-PCR analysis kit.

24. A kit as claimed in claim 21 for detecting PRV-1 mRNA, characterized in that it is a Northern blot kit.

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Abstract

This document describes a nucleotide sequence which encodes the PRV-1 protein, and essentially comprises the sequence ID No. 1, and also a process for detecting this gene [lacuna] the mRNA coded by this gene and the polypeptide encoded by this gene.

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Fig. 1

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PASQHEGGGAEGLESLTWGVGLALAPALWWGVVCPSC

Fig. 2

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below, next to my name.
I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled The PRV-1 Gene and Use Thereof the specification of which:

____ is attached hereto.
__X__ was filed on September 29, 2000
as United States Application Number _____
or PCT International Application Number PCT/EP00/09594

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above. I do not know and do not believe that the claimed invention was ever known or used in the United States of America before my invention thereof, or patented or described in any printed publication in any country before my invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, and that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (for a utility patent application) or six months (for a design patent application) prior to this application.

I acknowledge the duty to disclose all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d), of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
19947010.3 (Number)	DE - Germany (Country)	30-September-1999 (Day/Month/Year Filed)	X Yes	____ No
PCT/EP00/09594 (Number)	WO - PCT (Country)	29-September-2000 (Day/Month/Year Filed)	X Yes	____ No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	____ Yes	____ No

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below

_____ (Application Number) _____ Filing Date

_____ _____

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Number)	Filing Date	(Status – patented, pending, abandoned)
(Application Number)	Filing Date	(Status – patented, pending, abandoned)
(Application Number)	Filing Date	(Status – patented, pending, abandoned)

I hereby appoint Toni-Junell Herbert, Registration No. 34,348, Mark R. Shanks, Registration No. 33,781, Joseph G. Contrera, Registration No. 44,628, Shelly Guest Cermak, Registration No. 39,571, Christina M. Gadiano, Registration No. 37,628, Chris Aniedobe, Registration No. 48,293, Kristin Vidovich, Registration No. 41,448, and Carrie Stroup, Registration No. 50,172 of SHANKS & HERBERT, telephone (703) 683-3600, with a mailing address at:

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Alexandria, VA 22314

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith.

The undersigned hereby authorizes the U.S. Attorneys named herein to accept and follow instructions from undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

Full Name of Sole/First Inventor: PAHL, Heike

Inventor's Signature: Heike L. Pahl Date: 26.3.02

Residence: Freiburg DEX Citizenship: Germany
(City, State) (Country)

Post Office Address: Spargelweg 32, 79112 Freiburg, GERMANY

SEQUENCE LISTING

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